

PCT/SE2003/001077

Amended claims 2004-09-14

## CLAIMS

1. A DNA-library for production of a library of double stranded RNA-molecules (dsRNA) of a predefined length, the library consisting of double stranded DNA-molecules (dsDNA) where each dsDNA comprise a stretch wherein both strands contiguously encode a promoter, a dsRNA-encoding sequence of 10-30 base pairs encoding the dsRNA to be produced and a transcription termination sequence, wherein each of said promoters has been mutated to include the sequence complementary to the termination sequence of the other strand.
2. A DNA-library according to claim 1, wherein said promoters are H1-promoters or U6-promoters that have been mutated so as to incorporate an AAAAAA-stretch at the end of the promoter, immediately next to the transcription starting site.
3. A DNA-library according to claim 1 or 2, wherein said dsRNA-encoding sequence is randomized in between 4 and all positions.
4. A DNA-library according to any of claims 1-3, wherein the produced dsRNA contains a single stranded region at one end.
5. A DNA-library according to any of claims 1-3, wherein the produced dsRNA contains single stranded regions at both ends.
6. A DNA-library according to claim 4 or 5, wherein at least one of the single stranded regions of the dsRNA is a poly-U overhang.
7. A DNA-library according to claims 4 or 5, wherein at least one of the single stranded regions of the dsRNA is a UU overhang.
8. A DNA-library according to any of claims 1-7, wherein it is constructed in a plasmid vector.
9. A DNA-library according to any of claims 1-7, wherein it is constructed in a viral vector.
10. A DNA-library according to any of claims 1-9, wherein the randomness of the library was modified by selection of the random DNA oligonucleotides, before cloning the said random DNA oligonucleotides into the vectors, through hybridization to a total RNA preparation or total mRNA preparation from a source, whereby only the oligonucleotides

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hybridized to the source RNA (or mRNA) are subsequently cloned into the vector, and wherein the source can be a cell, a cell line, a tissue, or a organism.

11. A kit containing the DNA-library according to any of claims 1-10.
12. An RNA-library obtained from the DNA-library according to any of claims 1-10.
13. A method of using the DNA-libraries of any of the claims 1-10, wherein the library is transiently or permanently introduced into cells as a mixture.
14. A method of screening for double stranded RNA with biological functions comprising the use of the DNA-library according to any of claims 1-10 or the RNA-library according to claim 12.
15. A method of screening for novel genes comprising the use of the DNA-library according to claims 1-10 or the RNA-library according to claim 12.
16. An individual DNA-member of the DNA-library according to any of the claims 1-10.
17. An individual RNA-member of the RNA-library according to claim 12.
18. Use of a DNA-molecule comprising the DNA-sequence  $AAAAA(N)_nTTTTT$ , wherein  $(N)_n$  is a randomized region of 19, 20 or 21 nucleotides, in the production of dsRNA-molecules.
19. An H1 RNA-polymerase III-promoter mutated to have an AAAAAA-stretch at the end of the promoter immediately ahead of the transcription starting site.
20. A plasmid with two mutated RNA polymerase III promoters, each embedding one transcription termination sequence for the other promoter, and a siRNA-encoding region between the promoters.

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